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TITLE: The Role of Histone Demethylase Jmjd3 in Immune-Mediated Aplastic Anemia

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Fort Detrick, Maryland 21702-5012

The text of the report must include all sections addressed in the table of contents to include the following. **DO** include the bolded section headings, but **DO NOT** include the *italicized* descriptions of section contents in your submitted reports.

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14. ABSTRACT
Acquired aplastic anemia (AA) is a condition of bone marrow failure (BMF) characterized by blood pancytopenia and BM hypoplasia. In most cases, AA is an immune-mediated disorder with destruction of hematopoietic stem and progenitor cells by T cells. There is increasing evidence that CD4+ T effector cells that produce high levels of IFN-γ are associated with AA in patients and experimental AA mice. IFN-γ displays potent effects on suppressing hematopoiesis. Immunosuppressive therapy with antithymocyte globulin in combination with cyclosporin A (CsA) can induce a hematologic response in about two-thirds of AA patients. However, relapse occurs in up to 35% of AA patients when CsA is withdrawn at 6 months. Allogeneic BM transplantation (BMT) has significantly improved the survival of AA. However, graft-versus-host disease (GVHD) remains a major barrier to the success of the procedure. Novel approaches are needed to improve the outcomes of AA treatment.

15. SUBJECT TERMS-

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a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Acquired aplastic anemia (AA) is a condition of bone marrow failure (BMF) characterized by blood pancytopenia and BM hypoplasia. In most cases, AA is an immune-mediated disorder with destruction of hematopoietic stem and progenitor cells by T cells. There is increasing evidence that $CD4^+$ T effector cells that produce high levels of IFN-γ are associated with AA in patients and experimental AA mice. IFN-γ displays potent effects on suppressing hematopoiesis. Immunosuppressive therapy with antithymocyte globulin in combination with cyclosporin A (CsA) can induce a hematologic response in about two-thirds of AA patients. However, relapse occurs in up to 35% of AA patients when CsA is withdrawn at 6 months. Allogeneic BM transplantation (BMT) has significantly improved the survival of AA. However, graft-versus-host disease (GVHD) remains a major barrier to the success of the procedure. Novel approaches are needed to improve the outcomes of AA treatment.

The long-term goal of our studies is to develop novel approaches to control inflammatory T cells causing BMF and GVHD. The objective of this application is to define the role of Jmjd3, which is a histone demethylase, in regulating inflammatory T cell responses, and identify an optimal approach to reduce BMF and GVHD by inhibiting Jmjd3. The **rationale** of these studies is that if we identify the critical roles of JMJD3 and its-regulated mechanisms in BM-destructing T cells, we can further define optimal therapeutic approaches to modulate inflammatory T cell responses for controlling AA. These studies are **highly significant** because they would potentially lead to novel and clinically relevant strategies to improve the outcomes of therapy for AA, and may have broad implications in other T cell-mediated disorders such as autoimmune diseases and chronic infection.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

2.1. Aplastic anemia
2.2. T cells
2.3. Th1 cells
2.4. Th17 cells
2.5. MDSC (Myeloid derived suppressive cells)
2.6. Jmjd3
2.7. Utx
2.8. GSK-J4
2.9. Ezh2
2.9. H3K27me3
2.10. Graft-versus-host disease (GVHD)
2.11. Bone marrow transplantation
2.12. NSG mice
2.13. xGVHD
2.14. Hematopoietic stem cells (HSCs)

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.*

- **What were the major goals of the project?**
  - *List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project identify these dates and show actual completion dates or the percentage of completion.*

The major goals of the project include: **1)** understanding the roles of JMJD3 in the generation and maintenance of BM-destructive T cells. Understanding the cellular mechanisms of JMJD3 action in inflammatory T cells is essential to establish how BMF-mediating T cells develop and persist during AA process; and **2)** assessing whether JMJD3 and Ezh2 cooperatively regulate T cell production of IFN-γ in AA mice. Identifying the molecular mechanisms by which JMJD3 and its-counteracting enzyme Ezh2 orchestrate transcriptional programs (such as T-bet) for inducing and sustaining T cells mediating BMF.

- **What was accomplished under these goals?**
  - *For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.*

During the past year of support, the **major activities** of our research focus on: **A)** establishing the animal protocols and IRB protocol; **B)** investigating the impact of blocking chromatin enzymes that modify trimethylation of H3K27me3 in T cells; and **C)** developing new approaches to modulate BM-disrupting T cells.

Our **objectives** are to identify: **1)** the roles of JMJD3 in marrow-destructing T cells; and **2)** JMJD3-regulated molecular pathways that mediate alloreactive T cell responses. Results from these experiments will be important for developing novel approaches to reduce BMF and GVHD by targeting Jmjd3-mediated pathways in alloreactive T cells.

Our **significant finding** is the discovery of an important epigenetic regulation pathway, which is initiated by Jmjd3 removal of the repressor H3K27me3 and involves a Ezh2-orchestrated transcriptional programs. This pathway may help our understanding of epigenetic regulation of T cell inflammation and development of novel approaches to modulate BMF andGVHD.

Accomplishments:

The major accomplishment over the past year is the identification that Jmjd3 may play a key role in initiating a cascade reaction of epigenetic effects on regulating effector differentiation during antigen-driven T cell responses. This epigenetic pathway may represent an effective target for modulating inflammatory T cells that disrupt BM cells.

A). Background. JMJD3 is a histone demthylase that mediates demethylation of H3K27me3 and acts primarily as a gene activator.[32,33] Therefore, **JMJD3 regulates genes that are targeted by Ezh2-mediated H3K27me3 in an opposite manner**.[32-35]

Interestingly, a recent study identifies the importance of JMJD3 in regulating $CD4^+$ T cell differentiation.[36] JMJD3 ablation does not affect thymocyte development but inhibits $CD4^+$ Th1 differentiation and T cell-mediated autoimmne colitis and experimental encenphalitis.[36,37] However, whether JMJD3 is critical for the proliferation and differentiation of human effector T cells, and whether JMJD3 can be targeted to modulate T cell-mediated BMF in AA mice, remain unexplored. **Our idea is that blocking JMJD3 represents a novel and therapeutic approach to control T cell-mediated BMF**.

Since we only recently acquired the approval from Human Research Protection Office Office of Research Protections US Army Medical Research and Materiel Command (A-19326 HRPO Approval Memorandum (Proposal Log Number BM150110, Award Number W81XWH-16-1-0055), our research activities for studying the role of Jmjd3 in T cells focus on the utilization of experimental mice.

B). JMJD3 is a down-stream mediator of TCR signaling in human T cells. We want to investigate the upstream signals that may be important for activating Jmjd3 to reduce H3K27m3.

Stim1 mediates Ca^{2+} signals, and is triggered by TCR ligation and antigen activation. Stim1 can activate and NFκB pathways to drive T cell proliferation and differentiation (**Fig.1A**). We found that Jmjd3 was rapidly induced in activated CD8 T cells within 2hours of TCR ligation, further increased by 4 hrs and peaked by 6hrs. Loss of Stim1 resulted in lower levels of Jmjd3 and decline of Jmjd3 at a faster rate, with significantly reducing by 6 hrs (**Fig.1A**). TCR activation induced Ezh2 in CD8 T cells at a slower rate than Jmjd3, peaked by 24 hours after activation (**Fig.1B**). Stim1 deficiency impaired the early induction of Ezh2 (by 6hrs), but did not significantly affect Ezh2 after 24 hours. Thus, upon TCR activation, Stim1-mediated signals may have differential roles in regulating Jmjd3 and Ezh2 expression and function.

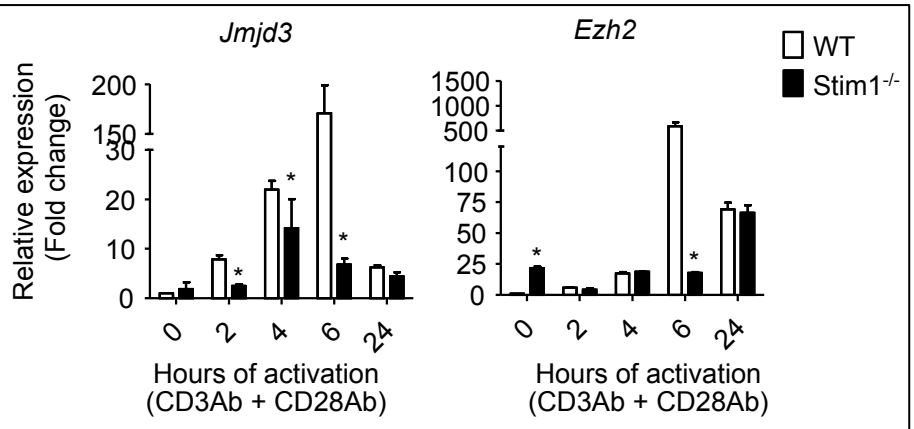

Fig.1. TCR ligation induces rapid uprgulation of Jmjdj3 and Ezh2 transcription in activated murine WT and Stim1-deficient ($Stim1^{-/-}$) $CD8^+$ T cells. Mouse $CD8^+$ T cells were activated by anti-CD3 Ab and ani-CD28 Ab over a period of 24 hours. RNA was extracted from these cells at indicated time points of culture. The expression of Jmjd3 and Ezh2 mRNA was measured using real-timeRT-PCR. *, $p<0.05$.

C). During effector differentiation, activated T cells progressively decrease cellular levels of transcription-repressive H3K27me3. Upon TCR-ligation, $CD8^+$ T cells were activated to upregulate the expression of activation markers CD25 and CD69, produced $CD62L^{hi}$ central memory precursor T cells (T_{CMP}) and $CD62L^{lo}$ effector T cells (T_{EFF}) (**Fig.2A**). T_{CMP} had higher levels of cellular H3K27me3 than T_{EFF}, and T_{EFF} can be

classified into at least two cell subsets: H3K27m3^{hi} T_{EFF} and H3K27m3^{lo} T_{EFF} (**Fig.2B**). Intracellular cytokine assay showed that T_{CMP} produced 10-fold less in frequency of IFN-γ and TNF-α than T_{EFF} (**Fig.2C**), suggesting that the production of effector cytokines is associated with the reduction of H3K27m3. **Thus, removal of H3K27me3 repressor is an important step for activated T cells to undergo effector differentiation.** However, whether H3K27m3^{lo} T_{EFF} may produce higher levels of IFN-γ than H3K27m3^{lo} T_{EFF} is unclear. Further understanding how decreasing H3K27me3 causes cytokine production will be important to define the e pigenetic mechanism that regulates effector differentiation and memory formation during BMF.

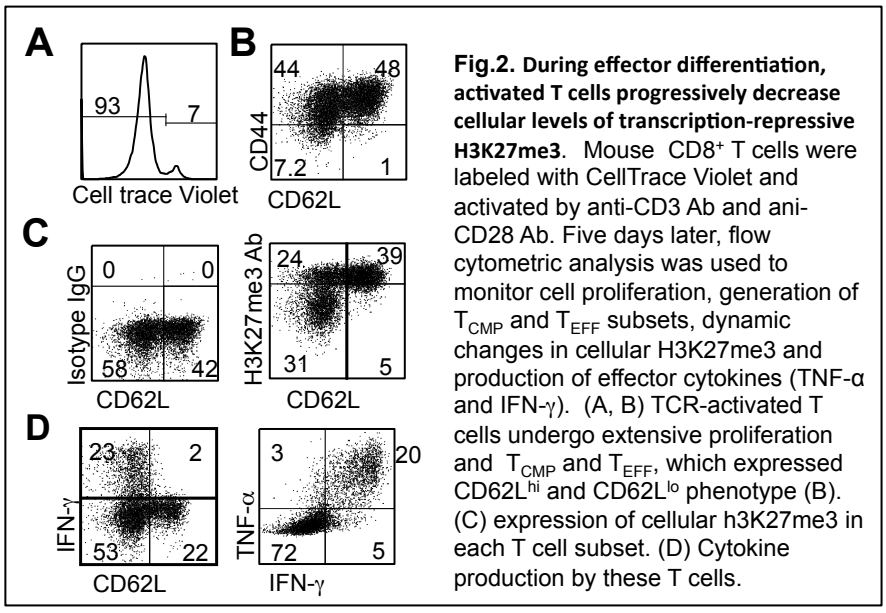

Fig.2. During effector differentiation, activated T cells progressively decrease cellular levels of transcription-repressive H3K27me3. Mouse CD8$^+$ T cells were labeled with CellTrace Violet and activated by anti-CD3 Ab and ani-CD28 Ab. Five days later, flow cytometric analysis was used to monitor cell proliferation, generation of T_{CMP} and T_{EFF} subsets, dynamic changes in cellular H3K27me3 and production of effector cytokines (TNF-α and IFN-γ). (A, B) TCR-activated T cells undergo extensive proliferation and T_{CMP} and T_{EFF}, which expressed CD62L^{hi} and CD62L^{lo} phenotype (B). (C) expression of cellular h3K27me3 in each T cell subset. (D) Cytokine production by these T cells.

D). Inhibiting Jmjd3 results in differential roles in induction of H3K27m3 and cytokines between T_{CMP} and T_{EFF}. T_{CMP} expressed higher levels of Jmjd3 than TEFF (**Fig.3A**). Addition of the Jmjd3 inhibitor GSK-J4 significantly increased the cellular H3K27me3 in T_{CMP} and H3K27m3^{lo} T_{EFF} (**Fig. 3B**). Notably, GSK-J4 even increased the expression of Jmjd3 protein in T cells (**Fig. 3B**), suggesting that the function of Jmjd3 is indeed reduced. Intriguingly, it also increased cellular levels of H3K27me3 in T_{EFF} (**Fig.3B**). Inhibiting Jmjd3 using GSK-J4 also dramatically decreased cytokine production (**Fig.3B**). Further experiments should address the following issues: **1)** whether different subsets of activated T cells have distinct susceptibility to Jmjd3 inhibitor; **2)** whether Jmjd3 may have

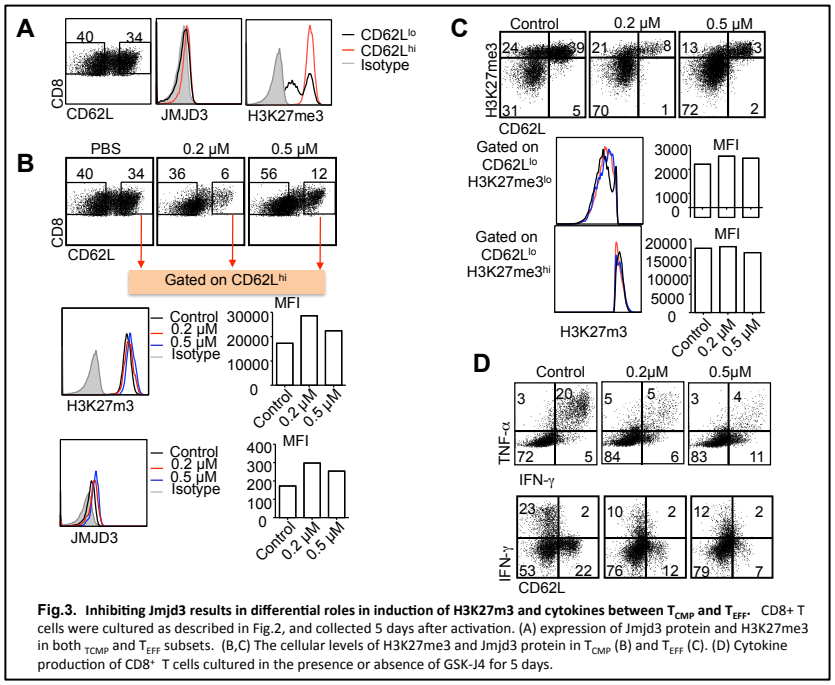

Fig.3. Inhibiting Jmjd3 results in differential roles in induction of H3K27m3 and cytokines between T_{CMP} and T_{EFF}. CD8+ T cells were cultured as described in Fig.2, and collected 5 days after activation. (A) expression of Jmjd3 protein and H3K27me3 in both T_{CMP} and T_{EFF} subsets. (B,C) The cellular levels of H3K27me3 and Jmjd3 protein in T_{CMP} (B) and T_{EFF} (C). (D) Cytokine production of CD8$^+$ T cells cultured in the presence or absence of GSK-J4 for 5 days.

differential roles in T cells at different differentiation stage; and **3)** whether GSK-J4-mediated reduction of cytokine may be associated with increased levels of H3K27me3 in T_{EFF}. Results from these experiments will clearly establish the correlation between H3K27m3 down-regulation and cytokine induction and effector differentiation.

E). GSK-J4 reduces T cell responses driven by allogeneic dendritic cells (DCs). We also tested the effect of pharmacologic JMJD3 inhibition on murine T cells activated by allogeneic DCs. T cells were labeled by fluorescence dye CFSE, which allowed us to track cell division history and proliferation. In this culture, dividing ($CFSE^{low}$) T cells represent mostly alloantigen-activated cells, whereas non-dividing ($CFSE^{high}$) T cells contain alloantigen non-responding cells[41]. Addition of GSK-J4 to the culture at day 0 of stimulation reduced the fraction of $CFSE^{low}$ T cells (**Fig.4A**), without significant effect on $CFSE^{high}$ T cells (**Fig.4B**). Thus, **GSK-J4 causes selective reduction of alloantigen-activated T cells**. GSK-J4 treatment also caused a significant decrease in frequency of IFN-γ-producing T cells (**Fig.4C**), confirming the importance of JMJD3 in Th1 cells.[36,37] Furthermore, GSK-J4 also reduced the fraction of IFN-γ-secreting CD8 T cells, elucidating the importance of JMJD3 in CD8 T cell responses, which has not been previously explored.

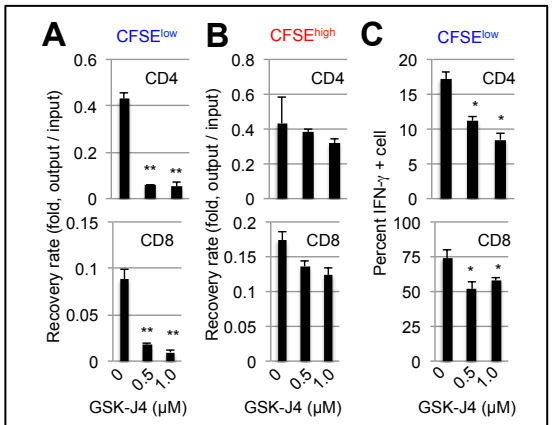

Fig.4. GSK-J4 inhibits alloreactive T cell responses in cultures. CD4 and CD8 T cells from B6 mice were stimulated with allogeneic DCs derived from BM of BALB/C mice. Five days later, cells were harvested to measure their proliferation and cytokine production. **(A) The fraction of** $CFSE^{low}$ **T cells. (B) The fraction of** $CFSE^{high}$ **T cells. (C)** Percent IFN-γ -producing cells. *, P<0.05; **, P<0.01.

Building on these results (Fig.1-Fig.4) and results from our unpublished studies (Nature Immunology, Revision, 2017, data not reported here), we propose an epigenetic regulation model that controls T cell immune responses during BMF and GVHD (**Fig.5**). Upon TCR ligation, activated T cells rapidly induce Jmjd3 and its-mediated removal of H3K27me3, leading early transcription of Tbx21 and production of Ifng. IFN-γ binds to IFNGRs to trigger IFNG signaling and subsequent activation of Akt. Active Akt mediates phosphorylation of Ezh2, dissociates Ezh2 from the promoter regions of major TFs (e.g., Tbx21, Prdm1, Eomes, Id2 and Id3), mediating durable reduction of Ezh2 function and subsequently exemplified effector differentiation.

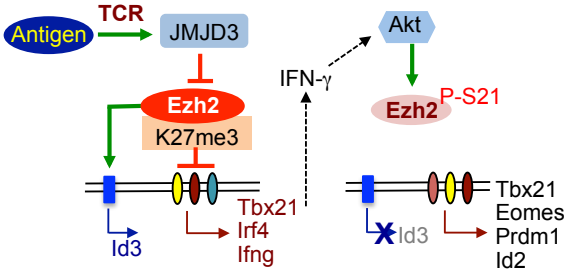

Fig.5. Hypothetical epigenetic regulation model that controls T cell immune responses during BMF and GVHD

In summary, we propose that better defining in depth this epigenetic regulation pathway, which is initiated by Jmjd3 removal of the repressor H3K27me3, followed by Akt-phosphorylation dissociated Ezh2 and activation of transcription factors for promoting effector differentiation, will lead to new strategies to control BMF and GVHD. Studies for next supporting time period will focus on illuminating this pathway. If this is

experimentally validated, it will open new perspectives to improve the efficacy of treating BMF and GVHD by targeting multiple key regulatory points.

- **What opportunities for training and professional development has the project provided?**
  - *If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."*
  - *Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.*

Nothing to report

- **How were the results disseminated to communities of interest?**
  - *If there is nothing significant to report during this reporting period, state "Nothing to Report."*
  - *Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.*

Nothing to report

- **What do you plan to do during the next reporting period to accomplish the goals?**
  - *If this is the final report, state "Nothing to Report."*

- *Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.*

Nothing to report

4. **IMPACT:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

- **What was the impact on the development of the principal discipline(s) of the project?**
  - *If there is nothing significant to report during this reporting period, state "Nothing to Report."*
  - *Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).*

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- **What was the impact on other disciplines?**
  - *If there is nothing significant to report during this reporting period, state "Nothing to Report."*
  - *Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.*

Nothing to report

- **What was the impact on technology transfer?**
  - *If there is nothing significant to report during this reporting period, state "Nothing to Report."*
  - *Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:*
    - *transfer of results to entities in government or industry;*
    - *instances where the research has led to the initiation of a start-up company; or*

- *adoption of new practices.*

Nothing to report

- **What was the impact on society beyond science and technology?**
  - *If there is nothing significant to report during this reporting period, state "Nothing to Report."*
  - *Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:*
    - *improving public knowledge, attitudes, skills, and abilities;*
    - *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
    - *improving social, economic, civic, or environmental conditions.*

Nothing to report

5. **CHANGES/PROBLEMS:** *The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

- **Changes in approach and reasons for change**
  - *Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.*

N/A

- **Actual or anticipated problems or delays and actions or plans to resolve them**
  - *Describe problems or delays encountered during the reporting period and actions or plans to resolve them.*

There is delay for setting up studies using human immune cells because of delayed approval of our IRB protocol. We will try to catch up during the next time period.

There is also delayed for setting up Jmjd3-conditional knock out mice because of unexpected mouse contamination in the institution who planned to make transfer. We will get these mice from the Jackson lab from May this year and will try to catch up the research progress.

- **Changes that had a significant impact on expenditures**
  - *Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.*

    N/A

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
  - *Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.*

    N/A

- **Significant changes in use or care of human subjects:**

  N/A

- **Significant changes in use or care of vertebrate animals.**

  N/A

- **Significant changes in use of biohazards and/or select agents**

N/A

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**

  Report only the major publication(s) resulting from the work under this award.

  - **Journal publications.** *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Publications:

1. Qingrong Huang, Shan He, Yuanyuan Tian, Yuting Gu, Pan Chen, Changhong Li, Jiefang Huang, Yongnian Liu, Min Jin, Shaoyan Hu, Qing Tong, Anqi Ma, Jian Jin, Elizabeth Hexner, Henry Fung, Ran Reshef, **Yi Zhang (Correspondence author)** and Yanyun Zhang. Hsp90 inhibition destabilizes Ezh2 protein in alloreactive T cells and reduces graft-versus-host disease in mice. (Blood 2017 :blood-2016-08-735886; doi: https://doi.org/10.1182/blood-2016-08-735886).
2. Meng L, Hu S, Wang J, He S, **Zhang Y (Correspondence author)**. DLL4+ Dendritic Cells: Key Regulators of Notch Signaling in Effector T Cell Responses. Pharmacol Res. 2016 Sep 14. pii: S1043-6618(16)30873-8.

- **Books or other non-periodical, one-time publications.** *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Janaki Purushe and **Zhang Y (Correspondence author)**. Histone Methyltransferases and T Cell Heterogeneity. Book chapter in "T Cell Diversity and Function", Edited by Jonathan Soboloff and Dietmar Kappes (in printing, 2016).

- **Other publications, conference papers, and presentations.** *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

- **Website(s) or other Internet site(s)**

  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

  Nothing to report.

- **Technologies or techniques**

  Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

  Nothing to report.

- **Inventions, patent applications, and/or licenses**

  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

  Nothing to report.

- **Other Products**

  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

  - *data or databases;*

- *biospecimen collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report.

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

- **What individuals have worked on the project?**
  - *Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."*

No change.

***Example**:*

Name:	*Hongxing Sun*
Project Role:	*Postdoctoral fellow*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*unchanged*
Contribution to Project:	*Performing experiments, collect and analysis of data, writing paper*
Funding Support:	

8. **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

- *If there is nothing significant to report during this reporting period, state "Nothing to Report."*

- *If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.*

Nothing to report

- **What other organizations were involved as partners?**
  - *If there is nothing significant to report during this reporting period, state "Nothing to Report."*
  - *Describe partner organizations - academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) - that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.*

    Provide the following information for each partnership:
    - **Organization Name:**
    - **Location of Organization:** *(if foreign location list country)*
    - **Partner's contribution to the projec**t *(identify one or more)*
      - **Financial support;**
      - **In-kind support** *(e.g., partner makes software, computers, equipment, etc., available to project staff)*;
      - **Facilities** *(e.g., project staff use the partner's facilities for project activities)*;
      - **Collaboration** *(e.g., partner's staff work with project staff on the project)*;
      - **Personnel exchanges** *(e.g., project staff and/or partner's staff use each other's facilities, work at each other's site)*; and
      - **Other.**

Nothing to report

9. **SPECIAL REPORTING REQUIREMENTS**

- **COLLABORATIVE AWARDS:** *For collaborative awards, independent reports are required from **BOTH**the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to https://ers.amedd.army.mil for each unique award.*
- **QUAD CHARTS:** *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

Nothing to report

10. **APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc. Reminder: Pages shall be consecutively numbered throughout the report. **DO NOT RENUMBER PAGES IN THE APPENDICES.***

Nothing to report

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****ADDITIONAL NOTES:**

MARKING OF PROPRIETARY INFORMATION: Data that was developed partially or exclusively at private expense shall be marked as "Proprietary Data" and Distribution Statement B included on the cover page of the report. Federal government approval is required before including Distribution Statement B. The recipient/PI shall coordinate with the COR/GOR to obtain approval. **REPORTS NOT PROPERLY MARKED FOR LIMITATION WILL BE DISTRIBUTED AS APPROVED FOR PUBLIC RELEASE.** It is the responsibility of the Principal Investigator to advise the COR/GOR when restricted limitation assigned to a document can be downgraded to "Approved for Public Release." **DO NOT USE THE WORD "CONFIDENTIAL" WHEN MARKING DOCUMENTS. DO NOT USE WATERMARKS WHEN MARKING DOCUMENTS.**

Last Modified Date: 21-Sep-2015

Annual reports must provide a complete summary of the research accomplishments to date with respect to the **approved** Statement of Work. Journal articles **can be** substituted for detailed descriptions of specific aspects of the research, but the original articles **must** be attached to the report as an appendix and

appropriately referenced in the text. The importance of the report to decisions relating to continued support of the research cannot be over-emphasized. A report shall be submitted within 30 calendar days of the anniversary date of the award and yearly thereafter. A final report will be submitted upon completion of the research (see para b. below for additional requirements for a final report).

DoD Award W81XWH-16-1-0055 - Reminder - Report Due 28-MAR-17.

PI Name: ZHANG, YI.

Your Annual report covering research for this period is due in this office by 28-MAR-17.

Failure to meet the reporting requirements of your agreement with this Command may result in a recommendation to reconsider funding for future awards with your organization. To avoid such action, you should take immediate action to complete your report.

To obtain a copy of the format requirements and to view a sample cover, Standard Form 298 and table of contents, click on "Technical Reporting Requirements" at the U.S. Army Medical research and Materiel Command web site http://mrmc.amedd.army.mil/index.cfm?pageid=researcher_resources.technical_reporting.

*** IMPORTANT ***

When you are formatting your report you are given the option to select between:

Reports with the UNLIMITED distribution designation which will become accessible to the general public through the Defense Technical Information Center (DTIC) data repository

OR

Reports with the LIMITED distribution designation which will have access restricted to U.S. Government agencies only; reports containing unpublished, patentable, or proprietary information that are not ready for public release should be given the LIMITED distribution designation.

If you have questions or would like further clarification please contact your assigned science officer.

You are to send your report as a PDF file to https://ers.amedd.army.mil.

Point of contact for this action is Ms. Katie Boggs at 301-619-7328 or by email at katie.l.boggs.ctr@mail.mil.

Regards,
Katie Boggs
Technical Editor
Information Management
Fort Detrick, MD

Classification: UNCLASSIFIED
Caveats: NONE